

Protozoa parasitic in Termites.

Part II.—*Jænopsis polytricha*, n. gen. n. sp., with Brief
Notes on two New Species, *Jænopsis cephalotricha*
and *Microjænia axostylis*.

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With Plates 18—21.

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INTRODUCTION.

In an earlier paper (1) I described the morphology and life-history of the commonest protozoa, *Ditrichomonas termitis*, resident in the hind gut of an Indian termite, *Archotermopsis wroughtoni*, Desn. In the present paper I propose to consider in a similar manner other of these parasites, which have already been partially described by Imms (10) under the designation Species β . For reasons given on page 399 I propose to found the new genus *Jænopsis*, containing the two species *J.cephalotricha* and *J.polytricha*.

A third species has not been described by Imms, and on account of its similarity to *Microjænia hexamitoides* (Grassi) I have named it *Microjænia axostylis*.

These three species are not so common in the termite as *D. termitis*, but they appear to occur with equal frequency in the two sexes of all casts. *J. polytricha* and *J.cephalotricha* are, however, more prevalent than *M. axostylis*, which is a relatively rare animal.

METHODS.

The methods adopted in the study of the parasites were broadly the same as those described in my previous paper. The two most useful stains were Heidenhain's iron hæmatoxylin and Dobell's hæmatein described by him in 1914 (2). As regards fixatives, Schaudinn's fluid containing 4.5 per cent. acetic acid as recommended by Dobell and Jepps (3) and Bouin's fluid are excellent for general use. It was found, however, that both these fixatives affected the parabasal apparatus of *J. polytricha*, as was recorded in the case of *D. termitis*. In order to obtain good images of these bodies I employed Fleming's strong fixative from which acetic acid had been omitted as recommended by Gatenby (5). For an elaboration of this point reference should be made to my previous paper.

All attempts to obtain artificial cultures of the parasites were unsuccessful.

JÆNOPSIS POLYTRICHA: LIVING CONDITION.

In shape this animal is broadly pyriform with a much rounded anterior end. Measurements of a large number of individuals show that the length varies from 129–95 μ and the width from 47–77 μ . These values are slightly lower than those obtained by Imms, who gives 159–114 μ as the length range and 95–41 μ as that for the width.

About one-third of the distance from the anterior extremity there is developed a well-marked depression or groove, somewhat oblique in position and extending round the whole body, so as to separate the animal into distinct anterior and posterior portions or “head and body” regions (Pl. 18, figs. 1, 2, *G.R.*).

For a long time I thought it possible that this groove might represent a pharynx, but this view is untenable. If the animal is observed in a solution containing powdered carmine the particles which pass into the groove on one side of the body are always ejected in a very short time from the other side, and in many cases the granules can be seen travelling along the floor of the groove in an irregular manner. In no case have I found any evidence that the carmine was ingested or that the depression represented anything more than a furrow in the body-plasma. Further, if a pharynx were represented by the groove one would expect to find food-particles situated in or near to it: this has never been seen though hundreds of specimens have been examined; in all cases the food-particles—wood—are situated towards the posterior end of the body.

At the anterior end of the body there is a solid structure, probably supporting in function which I have termed the supporting structure or piece (Pl. 18, figs. 1, 2, *S.P.*). From it arise two long rods extending through about two-thirds of the body length. They are situated in the middle of the endoplasm and take a somewhat curved course (Pl. 18, fig. 2, *P.T.*).

When I thought that the groove mentioned above represented a pharynx I was inclined to regard these rods as pharyngeal supports, but research has demonstrated that they are totally different in nature and must be homologised with the bodies found in *Jœnia annectans* and termed by Grassi "il regolo" and "il collare." I prefer, however, to adopt the terminology of Janicki (11) and name them "parabasal structures." A more detailed account of the bodies will be found on page 401.

Running over the anterior two-thirds of the body are longitudinal striations, which form a right- and left-handed series (Pl. 18, fig. 2, *S.T.*). Each member of the series takes its origin in the supporting piece of the head, and pursuing a straight course passes over the groove; it then turns either to the right or left side for its remaining length. On account of this when the animal is viewed from the front or back (dorsal or ventral) surface two series of striations are seen diverging from each other in the middle line (Pl. 18, fig. 2), but when seen from the side the whole body-surface appears to be striated (Pl. 18, fig. 1).

At first sight the whole of the body seems to be covered with long flagella; more detailed observation shows, however, that the flagella are arranged in two definite series, which may be termed the "head" flagella and the "body" flagella series (Pl. 18, fig. 2, *H.F.*, *B.F.*).

The first of these series possesses a large number of flagella, arranged in a tuft recalling that found in *Jœnia annectans*. These flagella are about two-thirds as long as the body and when functioning act together in unison in a whip-like manner, thus serving as the main propulsive organ of the body. Their origin is in the supporting piece of the head (Pl. 18, figs. 1, 2).

The body series of flagella arise from granules situated on the striations, a more detailed account of which I will reserve for a later section of this paper (p. 389). In length the flagella are about equal to those of the head but extend in all directions over the body-surface, taking a backward course.

They are by no means as active as the head-flagella, moving but little when the animal is swimming but maintaining a slow undulatory action when it is at rest. It appears as though their function is similar to that of the undulating membrane of *D. termitis* described in my previous paper.

Owing to the length of the flagella and to the circumstance that they are placed near to the groove, it often appears as though some of them were actually inserted in it and were analogous to the peristomial or undulating cilia of the Ciliates. This is not the case, for detailed observation shows that there are no flagella actually in the groove and that the erroneous impression is due to the free ends wafting over it.

A well-developed axostyle is clearly visible in the living animal, arising from the supporting piece and passing between the parabasal structures to reach the posterior end of the body, from which it issues to terminate in a sharp point (Pls. 18, 19, figs. 1, 2, 3, 11, A.X.). Usually only a small portion of the structure is free from the body but occasionally a relatively large part is seen. I have never observed any retraction or lateral movement such as was described in the living, *D. termitis*.

MOVEMENT.

The animal is an exceedingly powerful swimmer as might be surmised from its flagellar covering. As I have mentioned above the main propulsive organ appears to be the head series of the flagella, acting in a whip-like way. The whole apparatus first bends down on one side of the body and then rapidly moves upward to the middle line, repeating the process almost immediately. In this way the body is drawn forwards and at the same time rolled over to that side opposite to the one to which the flagella curve themselves during action. Thus while swimming the animal is continually rotating on its longitudinal axis.

Owing to the parabasal structures being slightly curved it is

possible to distinguish arbitrarily a right and left side, and for convenience I have called the side towards which the convexity of the parabasal points the left one. It is interesting to note that the flagella usually bend towards this, the left side, and the rotatory motion of the animal is therefore from left to right—that is, clock-wise.

The remainder of the flagella scattered over the body have an entirely different action; they may be seen projecting in all directions from the body edge and are in continuous movement, which is, however, so erratic and feeble that it may be described as quivering. It is difficult to believe that they play any important part in the translation of the animal.

FEEDING.

The main food supply of *J. polytricha* is the food-particles on which the host has been living. In nearly every specimen examined some of the particles have been found in the endoplasm scattered irregularly in the posterior region to which they are confined.

No cytostome is present and I have been unable to detect the method by which the animal obtains its food. As is well known, this is an unsolved problem in the majority of the termite parasites. It has been suggested that the posterior region of the body is capable of producing pseudopodia which engulf the food (*Trichonympha*), but in *Jænoposis* I have never been able to find any evidence that this method obtains, although much time has been spent watching these animals in a fluid to which powdered carmine has been added.

In other experiments the termites were fed with carmine and then after two days killed and their rectal contents examined. Although many of the protozoa contained carmine granules, sometimes in numbers, in no case was it possible to detect the method by which ingestion had occurred.

In this connection a paper by Buscalioni and Comes (1a), which appears to have been overlooked by many observers, is

of interest. With this work I hope to deal in a future paper, where I shall discuss the possible relationship of the Protozoa to their termite host.

MORPHOLOGY.

(A) Striations and Flagella.

In stained preparations there can be seen no differentiation into ectoplasm and endoplasm, but there is a delicate membrane to the body sufficient in strength to prevent variation in the shape of the animal. The cytoplasm is in most cases granular, vacuolated protoplasm rarely being encountered. As already mentioned, the wood-particles are confined to the posterior region of the body and are not surrounded by a distinct food-vacuole.

In preparations stained by Heidenhain's method the striations are very clearly seen. Their general arrangement has been already discussed, but when stained a number of interesting points are revealed.

In the first place it is noticed that the anterior two-thirds of the body is raised into a series of ridges, having their origin in the supporting piece of the head, in a manner similar to that described by Hartmann (9) in the Form A of his *Trichonympha*, now referred by Grassi (7, 8) to the genus *Pseudotriconympha*.

Each ridge of *J. polytricha* continues for a short distance as a narrow line, which broadens considerably, until at its thickest portion it measures about $3.9\ \mu$. Towards its posterior extremity narrowing again takes place so that ultimately a short thick thread is seen similar to the anterior one (Pl. 18, fig. 3, *S.T.*). The whole complex reaches a length varying in different animals from 67.7 – $76.8\ \mu$.

The striations seen in the living animal are the sides of these ridges, which, narrowing at the anterior and posterior extremities, give the appearance of a single line. Thus in the middle of the body each pair of striations corresponds to one ridge. Reference to Pl. 18, fig. 4, which is an enlarged drawing of two ridges, will render clear the above account.

On the broad part of the ridges are situated numerous

deeply-staining granules from each of which one body-flagellum takes origin (Pls. 18, 19, figs. 4, 11, *B.G.*).

Transverse sections through the body show that these flagella are arranged on crests of the body-surface (Pl. 19, fig. 8), and not in depressions, as has been described so often in the Ciliates, where striations are so commonly found. Probably a delicate membrane covers the granules, though this was not observed in the sections.

It is possible that the activity of the flagella in *Jœnopsis* may have caused this raising of the portions of the body-surface, through the continuous tension which they exert. It is true that their movement is feeble but they are so numerous and so closely placed together that their combined effect must exert considerable force on the delicate surface of the body.

In view of this suggestion it is of interest that at those places where the flagella are most numerous, and where the tension is therefore greatest, there the ridges are broadest, while at the anterior and posterior narrow portions the flagella are few.

In the same way the large tuft of head flagella may have produced the broad papilla, which I have termed the head, by their continual pulling on the protoplasm.

These head flagella arise from numerous basal granules arranged in two series, one on each side of the supporting piece (Pls. 18, 19, figs. 5, 11). This structure stains deeply with iron-hæmatoxylin, but readily gives up its colour when suitably treated, leaving the basal granules deeply stained. This affinity for stain was also found by Grassi (8) to obtain in the complicated supports—"complicati sostegni"—of *Staurojœnina mirabilis*.

(B) Parabasal Bodies.

Also from the supporting piece there arise the two bodies which I have already mentioned as homologous to "il regolo and il collare" of *Jœnia*, and which I have termed the "parabasal threads." In stained preparations they colour

deeply and are not easily decolorised by the ordinary reagents used for this purpose. The length varies from 66·6–80·8 μ according to the length of the entire animal and they are slightly curved. Their shape is rectangular, with the long side of the rectangle much greater than the short one. Measurements of these bodies in transverse section gave the relation of the two sides as above 3·1. Towards their anterior end, however, the long side narrows, becoming almost entirely equal to the short one, thus giving the appearance of a thick thread about 1·6 μ in diameter (Pl. 18, figs. 3, 5, *P.T.*). The two bodies are not arranged in the same plane as that of the supporting piece but transverse to it, so that one of the bodies is always at a lower level than the other—usually 7 or 8 μ deeper in the body. Also they run through the middle of the plasma in no way superficially, so that in order to obtain a sharp focus of the deeper body it is necessary to lower the objective of the microscope 20–30 μ below the level required for body surface focus.

In making preparations the pressure of the cover-slip and glass causes flattening of the animal with the result that the parabasal threads come to lie practically in the same plane; during this process the lower one is rotated on its axis giving an appearance such as is shown in the figures, where the narrow side of the upper parabasal is seen, but the long side of the lower one.

Situated on the broad surface of these parabasal threads there are numerous rod-like structures arranged irregularly; their length being about 6·8 μ and their diameter about 1·2 μ . They are most numerous on the median and posterior extremities, but a few are found up to the point of origin of the supporting piece. On account of their situation their whole length is seen projecting outwards in all directions only on that thread whose short side is viewed—that is, the upper one, while in the lower one the rods are seen as small rounded structures (Pls. 18, 19, figs. 5, 9).

I think there is no doubt but that these rods represent "le figlioline" described in *Jœnia* by Grassi (6, 7).

A short discussion of the probable homologies of these bodies will be found on p. 401.

Finally I may mention that these rods are only distinctly seen in those preparations fixed with Fleming's fluid as modified by Gatenby, or by a fixative containing neither corrosive sublimate or acetic acid. In preparations fixed by fluids containing these substances the rods are very variable as regards their presence, and at all times they are very indistinct in outline. An exactly similar effect was found in *D. termitis*, as described in my previous paper, where the whole question is briefly discussed.

(c) Nucleus.

Between and at the base of the arms of the supporting piece the nucleus is situated (Pl. 18, figs. 3, 5). It is a rather large body measuring 10–16 μ and slightly oval in shape. On account of its close proximity to the deeply staining supporting pieces and the parabasals it is often difficult to obtain a clear picture of it unless the stain is greatly extracted from the rest of the body.

Bounding the nucleus there is a distinct membrane, immediately within which is a clear space measuring in a 10.4 μ nucleus 2.02 μ and in a 14.1 μ nucleus 3.4 μ . This space is entirely devoid of threads radiating into it from the central chromatin mass, which is not homogeneous but composed of a ground substance, probably plastin, in which numerous chromatin masses are embedded. They are not, however, sharply defined bodies, but irregular as regards shape and size with indistinct margins merging imperceptibly into the ground-work (Pls. 18, 19, figs. 5, 7). Such is the characteristic "resting" nucleus.

(d) Axostyle.

The chief points regarding this structure I have already indicated, so that there only remains to describe certain details revealed by the employment of stains. Capriciousness regarding the reaction to stains is shown here as in

D. termitis, for in some slides the axostyle can scarcely be seen at all, while in others treated in the same manner, as far as can be ascertained, it is a prominent feature of the body. On the whole Dobell's hæmatein stain seems to be the best for demonstrating the axostyle, though it cannot be claimed as absolutely reliable for this purpose any more than the many other stains which I have tried. Also the method of fixing the preparation does not appear to affect the result. However, in slides showing the axostyle it appears to take origin in some of the basal granules of the supporting piece (Pl. 19, fig. 11). In its course through the body it runs ventral to the nucleus, but does not become attached in any way to the nuclear membrane.

While in the living animal it appears as a hyaline structure, very similar to that of *D. termitis*, when stained it is different. In *D. termitis* the axostyle retains its hyaline appearance, and a series of granules are seen running down the centre. In *J. polytricha*, on the other hand, the axostyle appears uniformly stained with no trace of median granules. Also it is much narrower in relation to the animal's size, measuring only 2μ in diameter, which is approximately the size of the axostyle of *D. termitis*, a much smaller animal.

DIVISION.

(A) Possible Cause of Division.

The division stages of *Jænopsis* are not readily found, which is in sharp contrast to what obtains in *D. termitis*, where every preparation contains a large number of animals in various stages of reproduction. For a long time I never encountered any specimen of *J. polytricha* in this phase of its life cycle, and I was beginning to despair of being able to work out the cycle of the animal when in April, 1918, a specimen in which the nucleus was in an early stage of division was encountered. The slide containing this form was made from the rectal contents of a male soldier termite. In the following month a female worker was found in which the

parasites were reproducing themselves. In July and August, however, I obtained several slides from the sexes of all castes in which the protozoa were in what might be termed an epidemic of reproduction. Since that date until the end of December, when I had exhausted all my stock of termites, no other dividing forms were seen.

From the above it might be concluded that reproduction took place only during the warm months of the year, and that the higher temperature exerted some stimulus to reproduction in the parasites.

To test this hypothesis, during the months of October, November and December a few termites, representative of all castes, were placed in small boxes containing the wood on which they feed and incubated at a temperature of 90° F. The insects remained at this temperature for periods ranging from 3 days to 2 months. Throughout the entire period the insects remained in a perfectly healthy condition. Slides of their rectal contents were made at frequent intervals, usually twice a week, but in no case was there evidence of great reproductive activity. The table below gives the results of these experiments. It must be noted also that on killing the termites their protozoa appeared quite normal and in large numbers, showing that they had suffered no ill-effects from their changed conditions.

TABLE.

The word "negative" indicates that after examining the slides made of the rectal contents of the termite which had been killed no dividing *Jænopis* were found.

| | | | |
|---|----|----|----|
| Soldier ♂, killed after 3 days, negative. | | | |
| Nymph ♀ | .. | .. | .. |
| " ♂ | .. | .. | .. |
| Worker ♀ | .. | .. | .. |
| Soldier ♀ | .. | 5 | .. |
| Nymph ♂ | .. | 7 | .. |
| " ♀ | .. | 7 | .. |
| Worker ♂ | .. | 7 | .. |

| | | |
|-----------|-------------------------|-----------------------|
| Soldier ♂ | , killed after 10 days, | negative. |
| Nymph ♀ | " 14 " | " " |
| Worker ♂ | " 14 " | " " |
| " ♀ | " 14 " | one dividing form. |
| Soldier ♀ | , killed after 17 days, | negative. |
| Worker ♂ | " 21 " | " " |
| " ♂ | " 21 " | " " |
| " ♀ | " 21 " | " " |
| Nymph ♀ | , killed after 25 days, | one dividing form. |
| " ♂ | " 28 " | negative. |
| Worker ♀ | " 28 " | " " |
| " ♂ | " 28 " | " " |
| Soldier ♀ | , killed after 32 days, | negative. |
| Worker ♀ | " 35 " | " " |
| " ♂ | " 35 " | " " |
| " ♂ | " 38 " | " " |
| Nymph ♂ | " 56 " | three dividing forms. |
| " ♀ | " 56 " | negative. |

These experiments are by no means exhaustive, but I had not sufficient material to carry out my investigations on a large scale or to determine the action of any external factor other than that of temperature. The results obtained, however, are not without interest, and seem to indicate that temperature is not the operative factor in bringing about reproduction. My view is rather that the conditions necessary are resident in the animals themselves and are more or less independent of other factors. The reason, then, for my finding reproducing animals in the warm months of the year and not in the cold I would ascribe, either to my not being able to examine sufficient material during these latter months, or to ill-luck in not killing animals which were harbouring parasites undergoing division, which phase possibly is rapidly passed through.

(B) Division Phases.

The early stages of division are exceedingly difficult to find, and even in those slides in which the majority of the animals are reproducing it is almost impossible to find a specimen

showing the method by which the body organella become reduplicated. That they do so, however, at an early stage is evidenced by the many animals seen with a double set of these structures, but with the nucleus in the various stages of division (Pl. 19, figs. 10, 15). It is probable that the supporting piece of the head and the parabasal threads undergo longitudinal division. My positive evidence for this view is based on one animal only, of which (Pl. 19, fig. 9) is a drawing. Here the parabasal threads and supporting piece are divided, or just about to complete their longitudinal division. Support for the conclusion is also given by the fact that in all reproducing animals—at whatever stage—a double set of organella can be seen (Pls. 19, 20, figs. 10, 15, 16, 17, 20). If they had been produced as new outgrowth and not by division of the pre-existing structures one would expect to find the intermediate growth stages, as was the case in the chromatic base of the undulating membrane of *D. termitis*. Such stages have never been observed.

As regards the rod-like parabasal bodies situated on the threads I am unable to state their method of division. There is no evidence of their breaking into granules, which are subsequently absorbed as Grassi has described (6, 7) in *Jœnia annectans*.

Turning now to the nucleus the first stage of division consists in the resolution of the chromatin into a large number of round granules, the number of which appears to be indefinite, but ranging between 50 and 60. The plastin framework is also broken up into a cloud of tiny granules distributed throughout the whole nucleus (Pl. 19, fig. 11). Coincident with this process the nucleus increases in volume, reaching a diameter of 7.7μ .

The next stage consists in the aggregation of the granules to form thin rods whose number varies from 8 to 12 (Pl. 19, fig. 12).

These rods now separate one from another, an equal number passing to each end of the elongating nucleus in which no trace of spindle-fibres can be detected. A constriction in the mem-

brane and further elongation of the nucleus occur, and at the same time the plastin granules aggregate round the chromatin rods (Pl. 19, figs. 10, 13, 14).

Finally complete nuclear constriction takes place and the two daughter-nuclei remain connected together by a thin thread (Pl. 19, fig. 15).

At this stage the rods break into round bodies, variable in numbers, which become irregularly distributed through the nuclear substance (Pls. 19, 20, figs. 16, 17, 18, 19, 20). These bodies rapidly lose their individuality and the typical resting type of nucleus is formed.

A curious feature of the whole process is that one of the daughter-nuclei is usually a little further advanced in reconstruction than the other one (Pls. 19, 20, figs. 15, 17, 20).

The plastin component of the nucleus aggregates in the middle at an early stage in division (Pl. 19, fig. 13), and during the changes described above it becomes approximately equally distributed between the two nuclei, forming finally the ground-work of the new bodies (Pls. 19, 20, figs. 14-20). As the nuclei migrate one from the other the thread connecting them disappears, but the two independent nuclei still continue to separate. The organella of the body, already divided, follow the nuclei; thus finally two complete animals are produced only requiring that the plasma should constrict in order for them to lead an independent existence (Pl. 20, fig. 20).

A characteristic feature of the reproduction is that during the nuclear migration the organella undergo a rotation through 180° . During the early division phases they are similarly orientated (Pl. 19, fig. 10), but later rotation occurs as is shown in Pls. 19, 20, figs. 15, 16, 17, 20. It is of interest that Grassi found the same to obtain in *Jœnia annectans* (6, p. 251). "I due nuclei, le due aree flagellate con relative regoli, collari, ecc, quando i due individui stanno per separarsi; in molti casi, forse in tutti, si sono attontanti l'uno dall' altro di 180° ."

As the supporting pieces separate each carries with it a

portion of the striations and body flagella, so that in animals in this condition (Pl. 19, fig. 15) each half of the complex possesses about half the total number of striations, leaving the other half of the body naked.

It is probable that after the separation of the animals into two, the portion of the body devoid of striations forms them "de novo." It will have been noticed that during the nuclear division no division centre of any kind is formed, and there is no trace of blepharoplasts, centrioles or even the paradesmose found in *D. termitis*—in fact the whole process is an example of a simple mitotic division.

There remains to be described the origin of the daughter-axostyles. As I have pointed out in a previous work (2), the accounts of the formation of these structures are widely divergent from one another, and are impossible to reconcile unless one assumes that this organ in the various flagellates has different origins.

As there is no extra-nuclear spindle in *Jænopsis* it is obvious that the daughter axostyles cannot be formed from such a structure as has been described in other flagellates.

For *D. termitis* I gave reasons for believing that the axostyle underwent longitudinal division, and showed conclusively that the paradesmose could have no connection with it. This view is strengthened by the investigations in *Jænopsis*.

Whether, however, the new axostyles are derived from the existing one by splitting or whether they are developed "de novo" by growth from basal granules, as described by Kuczynski, I am unable to state with certainty.

Unfortunately an animal was never encountered in which the axostyles showed signs of division, but on the other hand there is no trace in any of the preparations of development by growth. In animals which have almost completed their reproductive phases two similar axostyles are always seen (Pl. 19, fig. 16). I therefore incline to the view that in *Jænopsis*, as in *D. termitis*, there is a rapid longitudinal division of the old structure.

CYST-FORMATION AND SEXUAL PROCESS.

Neither-cyst formation nor sexual process can be found in *J. polytricha*—a result which accords with previous investigation on termite protozoa. Probably Grassi supplied the reason for this when in 1911 he wrote: “Probalimenti la soppressioni dell’incistamento e for’anche quella della fecondazione sono rapportabili al costume or ricordato dei Termiti di mangiar la feccia dei propre compagni all’atto della emissione.”

GENERAL CONSIDERATIONS.

(A) Classification.

At the outset I must apologise for adding one more variation to the name *Jœnia*, which has already suffered much change at the hands of other workers. Thus we have *Microjœnia*, *Mesojœnia*, *Jœnina* and *Staurojœnina* founded by Grassi, to which Janicki (11) had added the genus *Parajœnia*. It may appear that I introduce unnecessary confusion to the literature by creating the genus *Jœnopsis*, but this name seems to me to express more clearly than any other the superficial resemblance that *Jœnopsis polytricha* has with *Jœnia annectans*. Grassi's (7) definition of the genus *Jœnia* is as follows: “Zona di flagelli richiamente la figure del nostro cuojo capelluto: pero non esattamente simmetrico ai due lati: bastoncello assile a mestolo, terminante posteriormente a oliva: doppio collare con figlioline: lamina e nastrino lettera esse evidentissimi, ciocondati da speciale lacune a piene di liquido: forme molto grandi.”

From the above it is clear that *Jœnopsis* is in broad outlines similar to *Jœnia*. Thus in both forms there is a tuft of flagella at the “head” end of the body, which arises from a supporting piece in *Jœnopsis* and from a flagellate area, crossed by transverse and longitudinal striations in *Jœnia*. In both animals there is a well-developed axostyle, though simpler in *Jœnopsis*, for there is no trace of the “mestolo” or “oliva” of *Jœnia*.

Probably, however, the most characteristic feature common to the two genera is the well-developed parabasal apparatus, consisting of parabasal threads to which the parabasal bodies (figlioline) are attached, the whole complex in *J. polytricha* strongly recalling the description given by Grassi of the "regolo," "collare" and "figlioline."

The quadrangular areas of protoplasm at the anterior end of *Jœnia*, termed by Grassi "lamina e nastrino a lettera," I have been quite unable to find in *Jœnopsis*, whose anterior protoplasm is perfectly homogeneous, and in this respect more nearly resembles the genus *Mesojœnia* where the structures are poorly developed. Further, there is no evidence in *Jœnopsis* of a lake filled with fluid such as described in *Jœnia*. Turning to other characters which separate *Jœnopsis* from *Jœnia*, its neighbouring genus, there may be mentioned the groove of the body. Of greater interest, however, are the longitudinal striations, with their attendant flagella of which I have written above.

The supporting piece of the head is also a characteristic feature, not found in *Jœnia*, but represented in the recently described genus, *Staurojœnina*, by a complicated body divided into four parts from which the anterior flagella arise. *Jœnopsis* and *Staurojœnina* are similar in lacking the definite blepharoplasts (batacchio) found in *Jœnia*. The genus *Parajœnia* (Janicki) appears to be widely separated from those mentioned above. The flagella are arranged in two curved lines at the anterior end, there is a trailing flagellum arising from a basal granule (blepharoplast), and the parabasal apparatus consists of two curved bodies each with its parabasal thread attached to the granule from which the trailing flagellum springs.

I would place *Jœnopsis* near to the genera *Jœnia* and *Mesojœnia* with affinities with *Staurojœnina*, but distantly connected with *Parajœnia*.

Using the classification of Grassi the family *Jœniidæ* would be composed of the following genera :

- (1) *Jœnia* (Grassi).
- (2) *Mesojœnia* (Grassi).
- (3) *Microjœnia* „
- (4) *Jœnia* „
- (5) *Staurojœnina* „
- (6) *Jœnopsis* (Cutler).
- (7) *Parajœnia* (Janicki).

(B) Parabasals.

In *Jœnia* Grassi has described a "regolo" and "collare" to which are attached small rods—the "figlioline." Janicki, to whom we owe the term "parabasal," homologises the "regolo" with the parabasal thread and the "collare" with its attached rods, as parabasal bodies. For the reasons given by Janicki I employ his nomenclature rather than that of Grassi. As I have pointed out already the parabasal apparatus of *Jœnopsis* is essentially similar to that of *Jœnia*, and I have therefore called the two long bands arising from the supporting piece the parabasal threads and the attached rods the parabasal bodies.

There are, however, points of difference between the structures in *Jœnia* and *Jœnopsis*, for in the former there is but a single parabasal thread which divides at its apex into two bands, one going to the blepharoplast and the other to the margin of the basal lamella of the flagella. The thread-like portion of the "collare" in *Jœnia* is but the continuation of the parabasal thread surrounding the axostyle. On this encircling band the parabasal bodies are attached. Thus Grassi includes in his term "collare" the parabasal bodies and the posterior part of the parabasal thread.

In *Jœnopsis* there are two threads, arising from the supporting piece. It seems probable that we have in *Jœnopsis* a condition more simple, for if the distal ends of the parabasal threads were joined together they would encircle the axostyle, and a further fusion of the proximal ends of the threads into a single band would produce a condition similar to that found in *Jœnia*. Further, the division of the

band at the anterior ends in this animal renders it probable that it is in reality composed of two threads fused together.

As regards the division of the parabasal bodies Grassi stated in his early paper (4) that they were reduplicated after the formation of the nuclear spindle, but was not certain whether they were developed "de novo" or by division, though he inclined to the latter view. In his later work (6) he described the bodies as usually being broken down into granules, which were absorbed and a new set of rods developed. In some cases, however, this does not occur, the whole apparatus being passed over to the daughter-organism and a new one developed in the old animal. As I have already stated the evidence in *Jœnopsis* is in favour of the view that the daughter parabasal bodies are produced by longitudinal division of the old ones as occurs with the parabasal threads.

In my earlier paper I have given a short account of the various modes of formation of the parabasal apparatus in the different genera of termite parasites; there only remains for me to point out that in *Parajœnia* the structures are very different in formation from what obtains in *Jœnia*, *Mesojœnia* and *Jœnopsis*, but that these three genera, on the other hand, broadly agree with the genus *Lophomonas*.

(c) Blepharoplast and Nuclear Division.

In both *Jœnia* and *Parajœnia* there is a well-marked body from which the parabasal threads arise together with the training flagellum in *Parajœnia* and the "sospensorio," etc., in *Jœnia*. In *Jœnopsis* no such body is found.

The chief interest in the nuclear division is the non-existence of any spindle structure, which is of importance in connection with the development of the axostyle. In my previous paper (2) reasons are given for doubting the view that the axostyles were always developed from the spindle, and *J. polytricha* supplies a further proof for my contention. Comparing the nuclear division of *Jœnopsis* with that of *Jœnia* it is at once evident that we are dealing with a simpler type. In

the latter animal there is a well-developed spindle, whose origin is not determined, but which ultimately takes up a position in a notch of the nuclear membrane. Elongation of the spindle occurs, and with it the chromatin is resolved into a skein, which splits transversely. Finally the nucleus divides and the daughter-bodies are attached for some time to the ends of the spindle. By further elongation the spindle becomes U-shaped, extending through the body, until in the end it splits into two to form a portion of the new axostyles.

It is obvious that as regards nuclear division there are few or no points of agreement between the two genera; and on account of the simpler nature of division, together with other points mentioned in the earlier part of the paper, I am inclined to regard *Jænopsis* as more primitive than *Jænia*.

DIAGNOSIS OF *JÆNOPSIS POLYTRICHA*.

A brief diagnosis of *J. polytricha* will be of use to students of termite protozoa and at the same time serve as a summary to this paper.

Jænopsis polytricha, n. gen. n. sp.—Flagellate of large size, the average being $112 \mu \times 62.3 \mu$. The animal is divided into two regions by a groove running somewhat obliquely round the body, thus demarcating a smaller anterior end from a larger posterior one. No cytostome is present. At the anterior end of the body there is found a supporting piece, with numerous basal granules from each of which a flagellum arises, the whole complex of flagella being arranged in a tuft, forming a whip-like locomotor apparatus.

The anterior two-thirds of the body is traversed by striations, arranged in a right- and left-handed series, taking origin in the supporting piece. These striations represent the edges of raised areas of the body surface, on which areas are situated basal granules, each with a flagellum.

Two parabasal threads, on which are developed numerous parabasal bodies, arise from the supporting piece, as does also a well-developed axostyle. The plasma is commonly granular and not differentiated into ectoplasm and endoplasm.

The nucleus is found between the two arms of the supporting piece, and is large, measuring from 10–16 μ in diameter. It possesses a well developed membrane with chromatin in the forms of irregular bodies embedded in a plastin ground-work. A clear space is seen between the chromatin and the peripheral membrane.

The method of reproduction is by simple division into two; no cyst-formation or sexual process has been observed.

The first stage of division is the reduplication of the supporting piece and parabasal apparatus; this probably takes place by longitudinal division of the existing bodies.

The nucleus divides by a simple mitosis with no development of spindle fibres or centrioles. Further, there is no formation of an extra spindle or paradesmose.

The origin of the daughter-axostyles is not clear, but the evidence points to the conclusion that the old axostyle divides longitudinally.

Habitat. Hind gut of the termite *Archotermopsis wrightoni* Desn.

Food. Particles of wood and cellulose.

JÆNOPSIS CEPHALOTRICHA, N. SP.

In the living condition it is at once apparent that this animal resembles *J. polytricha* in many respects, and I think that Imms (11) has included this species in his description of Species β . This is, however, incorrect, for *J. cephalotricha* lacks the body flagella of *J. polytricha*; and other characters, to be described below, show it to be a distinct species.

At the anterior end of the body there is a tuft of flagella (Pl. 20, figs. 21, 22 *H.F.*) which serve as locomotor appendages.

As in *J. polytricha*, the endoplasm contains a number of wood particles which are confined to the posterior end of the body. No sharp line of demarcation can be found between the ectoplasm and endoplasm, which is granular, though alveoli are by no means rare.

I have been unable to find a cytostome or the method by which food is ingested.

The groove so characteristic of *J. polytricha* is not developed, but the anterior end of the body is raised into a broad papilla-like process whose origin is probably similar to that of the same structure in *J. polytricha*.

As regards movement there is a difference between the two species: in *J. polytricha* progression is by a kind of gliding motion with continual revolution, due to the head flagella functioning to one side of the body. In *J. cephalotricha* the flagella alternate in their action from one side to the other, so that although the gliding movement is preserved there is no longitudinal revolution, but a slight oscillation from side to side.

Morphology.—*J. cephalotricha* is a smaller animal than *J. polytricha*, its length varying from 85–90 μ with a width of 45–60 μ , the average size being 87 μ \times 51 μ . Thus the shape is more or less oval with rounded ends.

At the anterior extremity there is a supporting piece (Pl. 20, figs. 21, 22), which shows the same reaction to stains as did the same structure in *J. polytricha*, and on each side there are numerous basal granules from which the flagella arise (Pl. 20, fig. 22).

From this supporting piece striations are given off which traverse about one-third of the body, and are developed all round it and not in a right- and left-handed series (Pl. 21, fig. 23).

Also these striations carry no basal granules or flagella. In *J. polytricha* the striations represent the edges of "hills" on which basal granules are placed, and every pair of striations joins together at the anterior and posterior ends of the body. In *J. cephalotricha*, on the other hand, the striations are simply thickenings of the body surface—in fact are comparable to those developed in the body of *Polymastix*. Probably they are strengthening structures and of totally different origin from those of *J. polytricha*.

Parabasal bodies and threads are not developed, but an

axostyle, similar to that of *P. polytricha*, takes origin in the supporting piece (Pls. 20, 21, figs. 21, 22, 24, 25).

The nucleus is situated at the anterior end of the body and is bounded by a distinct membrane, inside of which large chromatin masses are embedded in plastin. The diameter of the nucleus is about 5.7μ (Pl. 20, fig. 22).

Reproduction.—Unfortunately I have been unable to trace the life-cycle in this animal, though I have searched through the whole of my material. The only stages found are figured in Pl. 21, figs. 24, 25. In both these animals there are two nuclei, which have undergone reconstruction, and two supporting pieces with their flagella. This indicates that probably the reproduction is broadly similar to that of *J. polytricha*. There is no trace of any spindle structure, but this is not evidence that such a body does not occur—though by analogy with *J. polytricha* it is improbable—for, if formed, it may easily have disappeared as the stages in question are undoubtedly late ones.

MICROJENIA AXOSTYLIS, N. SP.

This animal is quite distinct from the two already described but is closely related to *Microjœnia hexamitoides* (Grassi).

The size is smaller than that of *J. cephalotricha*, ranging from $16.16-30 \mu$ in length, and $12.1-25 \mu$ in width.

At the anterior end there is a tuft of flagella arising from a series of granules arranged in two or three longitudinal rows (Pl. 21, figs. 26, 27).

The extreme anterior end of the body is not raised into a papilla, but is much thickened and flattened. A similar development is described by Grassi (6, 7), and he likens it to an operculum of a lake containing fluid. I have no evidence of the presence of any fluid in this region of the body; on the other hand I believe this thickening is an adaptation on the part of the animal to the strain produced by the vigorously functioning flagella. Whereas in *Jœnopsis* this strain was compensated by the supporting piece, here in *M. axostylis*

no such support is developed, but instead the ectoplasm is flat and thick.

The surface of the body is smooth with no trace of striations. The most distinguishable feature of the animal is the large axostyle arising from the anterior end of the body; and which, passing ventral to the nucleus, pierces the posterior end of the body surface. Its most noticeable feature is the width, which is $4.5-5\mu$, and which, relative to the body width, is enormous. Also it is hyaline in appearance with no affinity for stains, in this respect resembling the axostyle of *D. termitis*.

Unfortunately I have been unable to find any reproductive phases. Grassi (6) defines the genus *Microjœnia* as follows:

“Flagelli sorgenti in fitte serie sub-longitudinali da una zona vicina all'estremità anteriore delimitante perciò quivi un cerchio anteriore, il quale si presenta come l'opercolo di un'angusta lacuna contenente liquido, e risalta perchè sprovvisto di flagelli: bastoncino assile: una serie di speciali corpi tondeggianti al margine posteriore della zona suddetta: fore piuttosto piccole.”

It is obvious from the above that the animal I have described should be placed as a new species in the genus *Microjœnia*.

In conclusion I wish to express my thanks to Mr. J. B. Robinson for re-drawing for publication the figures which illustrate Pls. 18, 19, 20, 21, and to the Zoological Department, Manchester University, for a grant which enabled this work to be carried out.

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The papers marked * contain further references to the literature of Termite parasites.

EXPLANATION OF PLATES 18 to 21,

Illustrating Mr. D. Ward Cutler's paper on "Protozoa parasitic in Termites."

[All the figures, except figs. 1 and 2, are drawn from fixed and stained preparations. The optical apparatus employed was as follows: Zeiss apochromatic oil-immersion objective 2 mm. (N.A. 1.3) and compensating oculars 6, 12, 18. Critical illumination was always used. The method